

Supplemental information for A Thermodynamic Approach to PCR Primer Design

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P316 parameters

Here are the parameters used for Primer3.

- PRIMER_EXPLAIN_FLAG=1
- PRIMER_MISPRIMING_LIBRARY=
- PRIMER_PRODUCT_OPT_SIZE=250
- PRIMER_PRODUCT_MAX_TM=100.0
- PRIMER_NUM_RETURN=50
- PRIMER_MAX_END_STABILITY=9.0
- PRIMER_MAX_MISPRIMING=12.00
- PRIMER_PAIR_MAX_MISPRIMING=24.00
- PRIMER_MIN_SIZE=18
- PRIMER_OPT_SIZE=24

- PRIMER_MAX_SIZE=32
- PRIMER_MIN_TM=60
- PRIMER_OPT_TM=62.0
- PRIMER_MAX_TM=64
- PRIMER_MAX_DIFF_TM=3.0
- PRIMER_MIN_GC=20
- PRIMER_OPT_GC_PERCENT=50
- PRIMER_MAX_GC=80
- PRIMER_SELF_ANY=8.00
- PRIMER_SELF_END=3.00
- PRIMER_NUM_NS_ACCEPTED=0
- PRIMER_MAX_POLY_X=4
- PRIMER_OUTSIDE_PENALTY=0
- PRIMER_GC_CLAMP=0
- PRIMER_SALT_CONC=50.0
- PRIMER_DNA_CONC=50.0
- PRIMER_LIBERAL_BASE=1
- PRIMER_MIN_QUALITY=0
- PRIMER_MIN_END_QUALITY=0
- PRIMER_QUALITY_RANGE_MIN=0
- PRIMER_QUALITY_RANGE_MAX=100
- PRIMER_WT_TM_LT=1.0
- PRIMER_WT_TM_GT=1.0

- PRIMER_WT_SIZE_LT=1.0
- PRIMER_WT_SIZE_GT=1.0
- PRIMER_WT_GC_PERCENT_LT=0.0
- PRIMER_WT_GC_PERCENT_GT=0.0
- PRIMER_WT_COMPL_ANY=0.0
- PRIMER_WT_COMPL_END=0.0
- PRIMER_WT_NUM_NS=0.0
- PRIMER_WT_REP_SIM=0.0
- PRIMER_WT_SEQ_QUAL=0.0
- PRIMER_WT_END_QUAL=0.0
- PRIMER_WT_POS_PENALTY=0.0
- PRIMER_WT_END_STABILITY=0.0
- PRIMER_PAIR_WT_PRODUCT_SIZE_LT=0.05
- PRIMER_PAIR_WT_PRODUCT_SIZE_GT=0.05
- PRIMER_PAIR_WT_PRODUCT_TM_LT=0.0
- PRIMER_PAIR_WT_PRODUCT_TM_GT=0.0
- PRIMER_PAIR_WT_DIFF_TM=0.0
- PRIMER_PAIR_WT_COMPL_ANY=0.0
- PRIMER_PAIR_WT_COMPL_END=0.0
- PRIMER_PAIR_WT_REP_SIM=0.0
- PRIMER_PAIR_WT_PR_PENALTY=1.0
- PRIMER_PAIR_WT_IO_PENALTY=0.0
- PRIMER_INTERNAL_OLIGO_MIN_SIZE=18

- PRIMER_INTERNAL_OLIGO_OPT_SIZE=20
- PRIMER_INTERNAL_OLIGO_MAX_SIZE=27
- PRIMER_INTERNAL_OLIGO_MIN_TM=57.0
- PRIMER_INTERNAL_OLIGO_OPT_TM=60.0
- PRIMER_INTERNAL_OLIGO_MAX_TM=63.0
- PRIMER_INTERNAL_OLIGO_MIN_GC=20.0
- PRIMER_INTERNAL_OLIGO_MAX_GC=80.0
- PRIMER_INTERNAL_OLIGO_SELF_ANY=12.00
- PRIMER_INTERNAL_OLIGO_SELF_END=12.00
- PRIMER_INTERNAL_OLIGO_NUM_NS=0
- PRIMER_INTERNAL_OLIGO_MAX_POLY_X=5
- PRIMER_INTERNAL_OLIGO_MISHYB_LIBRARY=
- PRIMER_INTERNAL_OLIGO_MAX_MISHYB=12.00
- PRIMER_INTERNAL_OLIGO_MIN_QUALITY=0
- PRIMER_INTERNAL_OLIGO_SALT_CONC=50.0
- PRIMER_INTERNAL_OLIGO_DNA_CONC=50.0
- PRIMER_IO_WT_TM_LT=1.0
- PRIMER_IO_WT_TM_GT=1.0
- PRIMER_IO_WT_SIZE_LT=1.0
- PRIMER_IO_WT_SIZE_GT=1.0
- PRIMER_IO_WT_GC_PERCENT_LT=0.0
- PRIMER_IO_WT_GC_PERCENT_GT=0.0
- PRIMER_IO_WT_COMPL_ANY=0.0

- PRIMER_IO_WT_NUM_NS=0.0
- PRIMER_IO_WT_REP_SIM=0.0
- PRIMER_IO_WT_SEQ_QUAL=0.0
- PRIMER_TASK=pick_pcr_primers
- PRIMER_SEQUENCE_ID=
- PRIMER_PRODUCT_SIZE_RANGE=225-275
- PRIMER_FIRST_BASE_INDEX=1
- PRIMER_PICK_ANYWAY=1

Pythia parameters

Here are the input parameters to the pythia PCR module.

- Primer minimum length: 18
- Primer maximum length: 30
- Left primer minimum start positions in the input FASTA sequence: 0
- Left primer maximum start positions in the input FASTA sequence: 10
- Left primer minimum stop positions in the input FASTA sequence: 10
- Left primer maximum stop positions in the input FASTA sequence: 30
- Right primer minimum and maximum start positions in the input FASTA sequence: None
- Right primer minimum and maximum stop positions in the input FASTA sequence: None
- Minimum primer melting temperature:60
- Maximum primer melting temperature:64
- Targeted primer melting temperature:62

- Reaction annealing temperature: 60
- Minimum reaction equilibrium efficiency: 0.8
- Allowed bases at the primer 3' end: ACGT
- Allowed bases at the primer 5' end: ACGT
- Sodium concentration: 0.03
- Magnesium concentrations:0.001.
- Primer initial concentrations: 0.9E-6
- Target initial concentrations: 1E-16
- Minimum amplicon size: 225
- Maximum amplicon size: 275
- Maximum length of homopolymer runs in a primer: 4
- Maximum amplicon overlap for tiling applications: 25
- Whether to design nested primers: No
- If designing nested primers, the maximum nested amplicon offset: NA
- If designing nested primers, the maximum nested amplicon offset: NA
- Allowed bases at the primer 3' end of interior nested primers: NA
- Allowed bases at the primer 5' end of interior nested primers: NA
- Maximum size of non-specific amplicons to consider: 1000
- Maximum amount of time to spend on the primer design problem: See below

In the first round of parameter design, before the svm was developed, the maximum time per primer design iteration was set to 10 minutes, and then gaps were filled with 20 more minutes of computational effort per tiled region. In the second round of primer design, after introduction of the SVM, this was set to a very high default of 10000 seconds, but this amount of time was rarely if ever needed, due to the efficiency improvements attributable to the SVM.